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Lost in Translation

The first direct observation of individual ribosomes translating RNA yields information lost in bulk studies

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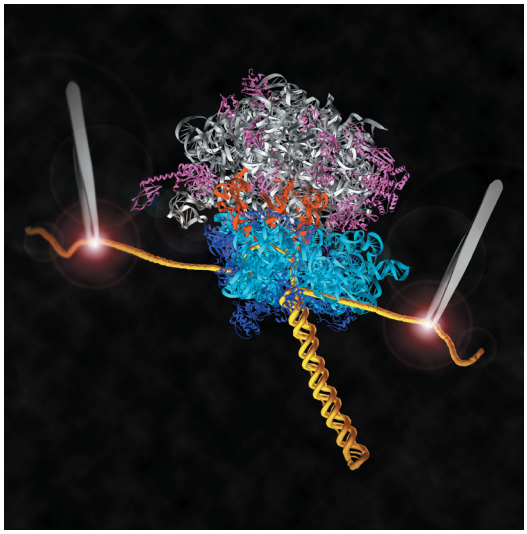
Of all the marvels inside living cells, few have remained more mysterious than the ribosome, the macromolecule that—in artists' conceptions—faintly resembles the gobs of chewing gum found under the seats of grade-school classrooms. Residing in a cell's cytoplasm, ribosomes are nature's equivalent of barcode scanners, directing the assembly of amino acids into proteins by translating the genetic instructions carried out of the nucleus by messenger RNA.

To date, what we know about translation dynamics has been based on "bulk" studies of trillions of ribosomes at a time. Now, for the first time, researchers have been able to study individual ribosomes in action. The results are surprising.

Using a combination of optical tweezers, microscopic beads, and specially engineered ribosomes, a multi-institutional team of researchers was able to follow a single ribosome as it translated a stretch of mRNA, one codon at a time (a codon is a triplet of nucleotides that codes for a specific amino acid). Contrary to conventional scientific wisdom, the ribosome's translation of mRNA was found not to be a continuous process.



Researchers used optical tweezers to follow the translation of a single mRNA (in yellow) by an E. coli *ribosome, one codon at a time. Translation was discovered not to be a continuous process but instead one that takes place through successive translocation and pause cycles. (Graphic by Laura Lancaster and Courtney Hodges.)*

Instead, the team discovered that translation takes place in a recurring cycle of step-pause-step movements as the ribosome translocates, or "steps," from one codon to the next. Each translocational step lasts less than a tenth of a second and is divided into three substeps, or pulses. Most of the pauses between steps last only a second or two (2.2 seconds was the average), but there are some pauses that last nearly two minutes. The discovery team believes that correlating these step-pause-step cycles to biochemical results gleaned from bulk studies should yield a wealth of new information about ribosomes and how they function.

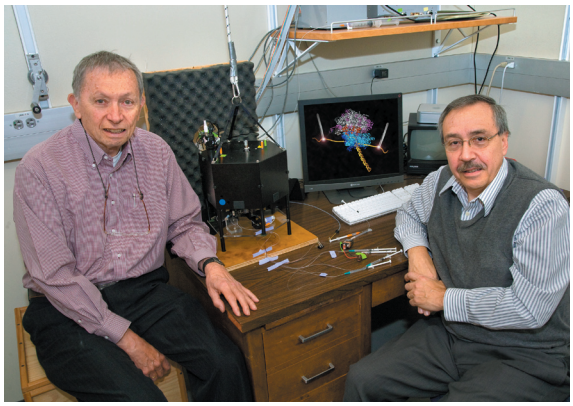
The research was led by biophysicist Carlos Bustamante and chemist Ignacio Tinoco, both of whom hold joint appointments with Berkeley Lab and the University of California at Berkeley, and by Harry Noller, a molecular biologist with UC Santa Cruz. The work, which was carried out under the auspices of the California Institute for Quantitative Biosciences (QB3), was reported in an article in the April 3, 2008 issue of the journal *Nature* and featured on its cover.

In addition to Bustamante, who is also a Howard Hughes Medical Institute investigator, Tinoco, and Noller, co-authors include the article's lead author Jin-Der Wen, a postdoctoral researcher in Tinoco's group; Courtney Hodges, a graduate student in Bustamante's group; Laura Lancaster, a postdoctoral researcher in Noller's group; Ana-Carolina Zeri of the Brazilian Synchrotron Light Laboratory; and Shige Yoshimura of Kyoto University.

"As is usually the case with single-molecule methods, the picture that emerges from these studies of individual ribosomes is quite different than what we had imagined from bulk studies," says Bustamante, a leading authority on the use of single-molecule visualization and manipulation techniques to study the dynamics, structure, and kinetics of molecular motors and nucleoprotein assemblies. "The existence of this translocation-pause pattern of events suggests that the mechanochemical cycle of the ribosomal machinery is segregated into two distinct phases. This discovery provides us with a window of opportunity for characterizing all the different processes that occur in each phase, and for establishing what is the crucial event that triggers the transition between the two."

continued

Tinoco, an expert on RNA and long-time collaborator with Bustamante, says that "Clearly there are many different chemical events taking place during the short pauses that precede the movement of the ribosome. These events—such as binding of the aminoacyl transfer RNA, binding of the EFTu-GTP, the peptidyl transfer reaction, etc.—must occur in order for the crucial event that couples chemical changes to actual ribosomal movement to happen. It is a huge surprise, though, to learn that during translation, the ribosome spends most its time waiting and not moving."



Ignacio Tinoco (left) is an expert on RNA. Carlos Bustamante is a leading authority on the use of single-molecule visualization and manipulation. The joint effort of these two long-time collaborators has yielded a wealth of information on RNA dynamics. (Photo by Roy Kaltschmidt, Berkeley Lab Public Affairs)

The ribosomes used in this study were engineered by Noller and his research group. Noller, who directs UC Santa Cruz's Center for Molecular Biology of RNA, is an internationally recognized expert on ribosomes. In 1999, he and his group used crystallography and x-ray beams from Berkeley Lab's Advanced Light Source to produce the first high-resolution images of a complete ribosome complex, a critical first step toward determining how ribosomes carry out their tasks.

For this latest study, Noller and Lancaster created an *Escherichia coli* ribosome attached to an mRNA molecule that was equipped with strands of DNA at each end to serve as tethers. Bustamante, Tinoco, Wen, and Hodges attached antibody-coated polystyrene beads to the DNA tethers and used laser beams as optical tweezers to exert opposing forces on each bead while the mRNA molecule was being translated. Through extension and force trajectories, the researchers were able to measure the time the ribosome spent at each codon, the number of mRNA nucleotides that moved through the ribosome during each translocation step, and the time required per step. The result was an unprecedented characterization of the dynamics of ribosome translation.

"This is probably the most difficult single molecule experiment ever attempted," says Bustamante. "We started working on this project more than six years ago as a collaboration between our three laboratories, and there were many years of frustration, false starts, and disappointment. Today, thanks to our stubbornness, we are very happy with the knowledge that many new discoveries lie ahead."

In their *Nature* paper, the researchers suggest that the three short pulses within each translocation step are evidence that the ribosome actually reads each individual nucleotide on a codon, rather than reading the codon's entire triplet of nucleotides at once; it's an idea that is anathema to current beliefs about the translation process.

As for the exceptionally long pauses between translocation steps, the team suspects this could be the formation of what is called a Shine-Dalgarno interaction between the mRNA and the ribosome, a common development in prokaryotic organisms like *E. coli*. They also speculate that the long pauses might be due to the ribosome's polypeptide exit channel getting clogged, or to regions of the mRNA that form secondary structures, which are difficult for the ribosome to read.

"Some of these long pauses end up becoming irreversible arrests," says Bustamante. "It is possible that these lengthy pauses are manifestations of spontaneous reading of frameshifts on the part of the ribosome."

The research team has now refined their technique so that for future studies they can exert and measure force on the ribosome as well as the mRNA, which should provide even more details about what is taking place during the translocational step-pause-step cycles.

Says Bustamante, "We must always keep in mind that in biology, every structure that exists is there to fulfill a function. Having the opportunity to endow these beautiful structures with movement and to rationalize their shapes and forms is an exciting task that is made possible only through single-molecule research. We have our hands full!"

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Additional information

"Following translation by single ribosomes one codon at a time," by Jin-Der Wen, Laura Lancaster, Courtney Hodges, Ana-Carolina Zeri, Shige H. Yoshimura, Harry F. Noller, Carlos Bustamante, and Ignacio Tinoco, appears in the 3 April 2008 issue of *Nature* and is available online to subscribers at http://www.nature.com/nature/journal/v452/n7187/abs/nature06716.html.

More information on the research of Carlos Bustamante can be found at http://alice.berkeley.edu/.

More about Ignacio Tinoco's research is at http://www.cchem.berkeley.edu/intgrp/tinoco.html.

More about the research of Harry Noller is at http://rna.ucsc.edu/rnacenter/noller_lab.html.

For more about QB3, the California Institute for Quantitative Biosciences, visit http://www.qb3.org/.